

REMARKS

Applicants have attached a marked version of the claims in this application. For the Examiner's convenience, Applicants have also attached a clean copy of all pending claims in this application after amendments.

Respectfully submitted,



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MARKED VERSION OF CLAIMS SHOWING AMENDMENTS

1. (Amended) Process for oxidising a substrate which is an acyclic or cyclic terpene [;] a or a cycloalkene or a substituted derivative thereof, and which process comprises: oxidising said [compound] substrate with a mutant haem-containing enzyme, the mutant comprising [the] a substitution of an amino acid in [the] an active site by an amino acid with a less polar side-chain.
3. (Amended) Process according to claim 2 in which the enzyme is one in which amino acid 47 [and/] or 51 or 47 and 51 of P450_{BM-3}, or amino acid 96 of P450_{cam}, or the equivalent amino acid[(s)] in a said homologue, have been changed to an amino acid with a less polar side-chain
6. (Amended) [An enzyme] The enzyme as defined in claim 4 excluding mutants of P450_{cam} which only have the mutations F87A-Y96G-F193A, F87A-Y96G-F193A- C334A, or T101M-T185F-V247M.
8. (Amended) A cell which expresses:
 - (i) a mutant haem-containing enzyme comprising [the] a substitution of an amino acid in [the] an active site by an amino acid with a less polar side-chain which in its naturally occurring form has an electron transfer reductase domain, or
 - (ii)
 - (a) a mutant haem-containing enzyme comprising [the] a substitution of an amino acid in [the] an active site by an amino acid with a less polar side-chain,
 - (b) an electron transfer reductase, and

- (c) an electron transfer redoxin; or
- (iii) (a) (1) P450_{cam}, or a fragment thereof; or
- (2) a naturally occurring homologue of P450_{cam} or a fragment thereof; or
- (3) a mutant P450_{cam}, or a mutant of a naturally occurring homologue of P450_{cam} comprising [the] a substitution of an amino acid in [the] an active site by an amino acid with a less polar side-chain; or
- (4) a P450_{cam} which has at least 70% amino acid homology with (1), (2) or (3) and optionally in which amino acid 96 has been changed to an amino acid having a less polar side-chain; and
- (b) an electron transfer reductase; and
- (c) an electron transfer redoxin; or
- (iv) (a) (1) P450_{BM-3}, or a fragment thereof; or
- (2) a naturally occurring homologue of P450_{BM-3} or a fragment thereof; or
- (3) a mutant P450_{BM-3}, or a mutant of a naturally occurring homologue of P450_{BM-3} comprising [the] a substitution of an amino acid in [the] an active site by an amino acid with a less polar side-chain

excluding an *E. Coli* DH5 α cell in which the only mutants of P450_{cam} which are expressed are amongst the following:

H₂N-P450_{cam}-TDGTSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H₂N-P450_{cam}-TDGTRPGPGPGPSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H₂N-P450_{cam}-TDGTRPGPGPGPGPGPSST-putidaredoxin reductase-TDGASSS
putidaredoxin-COOH,

H₂N-450_{cam}-putidaredoxin reductase-TDGASSS-putidaredoxin-PLEL-P450_{cam}-COOH.

9. (Amended) [A] The cell according to claim 8 in which (a), (b) and (c) or (b) and (c) are expressed together in the same fusion protein.
10. (Amended) [A] The cell according to claim 8 in which:
(b) is putidaredoxin reductase or a fragment thereof; and/or
(c) is putidaredoxin or a fragment thereof.
11. (Amended) Process for oxidising a substrate which is an acyclic or cyclic terpene, or a cycloalkene, or a substituted derivative thereof[;] , and which process comprises oxidising said [compound] substrate with a mutant haem-containing enzyme, the mutant comprising [the] a substitution of a first amino acid in [the] an active site by an amino acid with a less polar side-chain, wherein the [compound] substrate is oxidised in a cell according to claim 8.
13. (Amended) Process for selecting a mutant of P450_{cam} or P450_{BM-3}, or a homologue thereof, for its ability to oxidise a [particular] substrate, which process comprises screening a group of said mutants for their oxidation effect on the [particular] substrate.
14. (Amended) Process according to claim 13 in which the mutant is additionally selected for its ability to oxidise the [particular compound] substrate to [a] an [particular] oxidation product.

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